Commentary

Progress on canalization

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In the 19th century, when evolutionary biologists focused on whole organisms, development played a central role in evolutionary theory. For much of the 20th century, genetic models and explanations replaced development at the center of evolutionary thought. Connections to development resurfaced at mid-century (1, 2) and accelerated after 1975, fueled by influential books (3–5) and by a resurgence of interest in the role of phenotypes in evolution and in the tension between recent selection and historical constraint in the design of organisms. By the turn of the new millennium, development had again become a major evolutionary theme in two quite different but interestingly connected ways. Under the label of Evo–Devo, the tools of molecular genetics are used to explore deeply conserved developmental mechanisms that are active early in development and thought to shape the regulatory systems responsible for the conservation of basic body plans (6). Under the label of the Genotype–Phenotype Map, a variety of theoretical and experimental programs explore the mechanisms and processes that shape the expression of genetic variation in phenotypes in nonlinear ways (7).

Is there a natural bridge between these two research fields? Do the deeply conserved developmental systems that produce adult morphology also influence the expression of genetic variation in the traits they shape? Such a connection would point to part of one of evolution's long-sought Rosetta Stones: the mechanisms connecting macro- to microevolution. Building on work by A. Wagner (8), Siegal and Bergman (9) provide one of the first demonstrations of the plausibility of such a connection for a major component of the genotype–phenotype map: canalization.

Canalization, now a classic idea, was suggested independently by Waddington (1) and Schmalhausen (2). Schmalhausen argued that canalization resulted from stabilizing selection shaping developmental mechanisms to buffer the expression of traits, holding them near their optimal states despite genetic and environmental perturbations. Waddington suggested that if canalizing mechanisms could be disrupted, hidden genetic variation would be released. He claimed to have perturbed canalizing mechanisms with environmental treatments of developing fruit fly larvae and from the increased genetic variation in the treatments inferred the existence of canalization. Recently, Rutherford and Lindquist (10) for *Drosophila* and Quietsch *et al.* (11) for *Arabidopsis* (Fig. 1) have demonstrated that altering development with inhibitors of HSP 90 causes a release of hidden genetic variation. This has attracted great interest, for if the inference of canalization from the evidence of released genetic variation is correct, then at least one very concrete mechanism causing canalization would now be within sight.

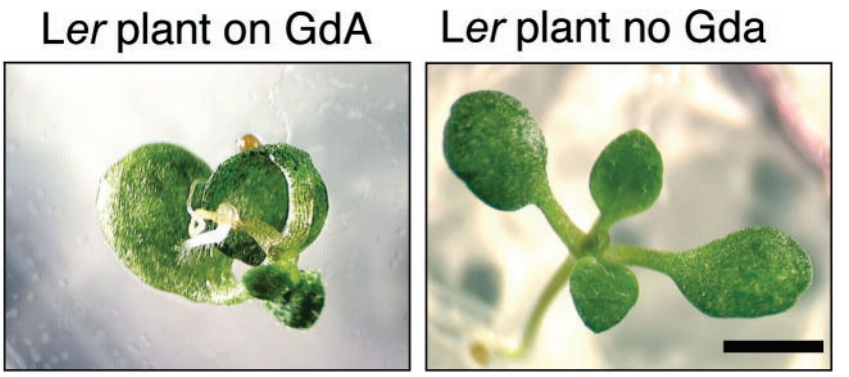

Fig. 1. The impact of geldanamycin on the L*er* accession of *Arabidopsis*. The plant on the left, treated with GDA, has an extremely curled hypocotyl and roots partially extended in the air. The plant on the right displays normal morphology. [Reprinted with permission of Quietsch *et al.* (11) (Copyright 2002, Nature Publishing Group, www.nature.com).]

However, the logical basis of that inference is not yet firm for several reasons. Waddington and Schmalhausen's original formulation of canalization has been remarkably difficult to define precisely in modern terms. Gibson and G. Wagner (12) express the essence of one problem: canalization describes a reduction in the expression of variation in phenotypes relative to some standard, but what standard? It has been difficult to control all of the effects that are plausibly involved to isolate a clear signal that could only have been produced by canalizing mechanisms. Another not entirely unrelated issue is that of unexplored alternatives: does the experimental release of hidden genetic variation necessarily demonstrate canalization because that is the only alternative, or is it simply consistent with canalization, leaving open plausible alternatives not yet eliminated? If so, what are those alternatives?

We now have a short but growing list of plausible alternative hypotheses that have not been rejected for mechanisms that reduce phenotypic variation and, when perturbed, release hidden genetic variation. These mechanisms do not necessarily have much to do with the original scenario for the evolution of canalization; they produce the appearance of canalization as a byproduct of other processes. Siegal and Bergman (9) describe one such mechanism. Because they build on an approach taken by A. Wagner (8), let us first consider his main result.

A. Wagner defined a phenotype as a state of gene activation and modeled evolution as changes in the strength of regulatory interactions among the genes. The elements of his model correspond to *cis*-acting transcription factors and to the genes whose expression they regulate. A. Wagner demonstrated that such regulatory networks acquire higher genetic robustness—better genetic canalization—under stabilizing selection. Similar results have since been found by several others. So far, so good: modern insights appear to be consistent with classical concepts.

Then a problem surfaced with the selection scenario thought to produce ge-

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netic canalization. Suppose that canalization evolves to buffer the phenotype against genetic perturbations caused by mutations. G. Wagner *et al.* (13) showed that the strength of selection for canalization increases with three variables: the intensity of stabilizing selection, the degree of canalization caused by the modifying allele, and the amount of genetic variation affected by the canalizing effect. However, at mutation–selection balance, the amount of genetic variation available for such a canalizing gene to work on will be reduced as the strength of stabilizing selection increases, for in eliminating genetic variation, stabilizing selection "eliminates the effects for which canalizing alleles are selected. . . Only with high mutation rates can genetic canalization be effectively selected in mutation-selection balance" (14). Because such high mutation rates ($>10^{-4}$ per locus) do not seem generally plausible, neither does the evolution of genetic canalization as a mechanism to buffer against the disruptive effects of mutations. If that is the case, then how do we account for the dramatic release of hidden genetic variation, now well anchored as an experimental result?

One possibility, the answer that Siegal and Bergman propose, is that phenotypic robustness to genetic perturbation has been wrongly interpreted as adaptive canalization. They assume selection against lethals, in their context genes that do not settle on a stable gene expression. Such selection evolves robustness against not only lethal mutations but also, as an unselected byproduct, against mutations of smaller effect that produce quantitative variation. It does so by causing networks of interacting transcriptional regulators to increase in complexity. Thus, more highly connected networks may be more canalized not because canalization of quantitative variation has been selected but because complexity in the underlying developmental network has been selected to suppress the effects of lethal disruptions of gene expression. Canalization is then a byproduct, and the release of hidden genetic variation is caused not by disruption of adaptive buffering mechanisms that evolved to conceal the genetic variation that is released, but by reduction in the complexity of regulatory networks that evolved to prevent the expression of lethal mutants.

If there is one mechanism that produces the appearance of canalization, might there not be others? Ancel and Fontana (15), working on RNA evolution, have shown that selection against environmental variation yields a response that includes genetic canalization as a byproduct. Thus, there are at least two byproduct mechanisms that have not yet been excluded, and there must be more. It may be that any developmental system that greatly reduces the dimensionality of phenotypes relative to the dimensionality of genotypes will produce the appearance of canalization by releasing hidden genetic variation when perturbed.

Does this all mean that the classical concept of canalization must be thrown out? Not necessarily. First let us return to the scenarios thought to select for canalization. Although mutations may no longer be a plausible stimulus for the evolution of genetic canalization, gene flow in a heterogeneous environment could still well be. Selection can produce quite different genetic states in each patch of a heterogeneous environment, and gene flow among patches can considerably reduce the degree of local adaptation to each patch. Such circumstances are plausibly frequent in natural environments, and would generate strong selection for genetic canalization to produce locally optimal phenotypes despite the input of foreign alleles. Thus, G. Wagner *et al.*'s (13) critique of the selection scenarios thought to produce genetic canalization, although correct as far as it goes, is—as they recognized—arguably not general, and adaptationists still have good reason to think that canalization may have evolved to keep phenotypes near local optima despite genetic perturbations. Nor must we accept that canalization is "just" a byproduct of increasing complexity of regulatory networks evolving for other reasons, for one of the reasons for the complexity of regulatory networks may in fact be the classical reason for canalization: to buffer phenotypes against genetic and environmental perturbation (8). Schmalhausen's stabilizing selection may be selection for complex regulatory networks.

Thus, here is the current state of play in canalization research. The experimental evidence of a release of hidden genetic variation when developmental systems are perturbed is consistent with classical selection scenarios for canalization, provided they are couched in terms of gene flow in heterogeneous environments and not in terms of mutations within a single population at equilibrium. Moreover, classical canalization would be sufficient to explain such evidence. The new alternatives, however, demonstrate that canalization is not necessary to explain the experimental results, and that we are therefore not forced to infer canalization from such evidence.

What sorts of experiments would forge a strong enough connection between ideas and evidence to compel ideas on canalization and phenotypic robustness to change when new evidence comes in? Experimental evolution recommends itself as a natural approach, but there is a lot of basic molecular developmental biology and genomics to be done before experimental evolution can be brought to bear effectively. We can hope for the day in the not-too-distant future when someone can say, "I know that this regulatory network of transcription factors and genes produces this trait in this well-understood way. In patchy environments with gene flow, I have placed this trait under strong stabilizing selection for a different optimum in each patch in one treatment, under weak stabilizing selection in another treatment, I have made it neutral in a third treatment, and I have placed it under disruptive selection in a fourth treatment. Here is how the regulatory network has changed over the course of many generations of well-controlled experimental evolution . . . ". The microbiologists will probably get there first, not only because of the shorter generation times and lower cost of replicates of bacterial populations, but also because they can ignore all of the hierarchical and modular complexity of multicellular development. However, the microbiological answer will only be generally convincing when it is demonstrated to hold up in multicellular eukaryotes. There is plenty to do.

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